Sucrose-Induced Accumulation of β-Amylase Occurs Concomitant with the Accumulation of Starch and Sporamin in Leaf-Petiole Cuttings of Sweet Potato

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ABSTRACT

β-Amylase of sweet potato (Ipomoea batatas L.), which constitutes about 5% of the total soluble protein of the tuberous root, is absent or present in only small amounts in organs other than the tuberous roots of the normal, field-grown plants. However, when leaf-petiole cuttings from such plants were supplied with a solution that contained sucrose, the accumulation of β-amylase was induced in both leaf and petiole portions of the explants. The sucrose-induced accumulation of β-amylase in leaf-petiole cuttings occurred concomitantly with the accumulation of starch and of sporamin, the most abundant storage protein of the tuberous root. The accumulation of β-amylase, of sporamin and of starch in the petioles showed similar dependence on the concentration of sucrose, and a 6% solution of sucrose gave the highest levels of induction when assayed after 7 days of treatment. The induction of mRNAs for β-amylase and sporamin in the petiole could be detected after 6 hours of treatment with sucrose, and the accumulation of β-amylase and sporamin polypeptides, as well as that of starch, continued for a further 3 weeks. In addition to sucrose, glucose or fructose, but not mannitol or sorbitol, also induced the accumulation of β-amylase and sporamin, suggesting that metabolic effects of sucrose are important in the mechanism of this induction. Treatment of leaf-petiole cuttings with water under continuous light, but not in darkness, also caused the accumulation of small amounts of these components in the petioles, probably as a result of the endogenous supply of sucrose by photosynthesis. These results suggest that the expression of the gene for β-amylase is under metabolic control which is coupled with the expression of sink function of cells in the sweet potato.

β-Amylase (α-1,4-glucan maltohydrolase, EC 3.2.1.2) is found only in plants and certain species of bacteria. Seeds of cereals and soybean contain a large amount of β-amylase, and β-amylase is also present in vegetative tissues of plants, for example, in leaves, roots, and cotyledons (27, 28). Although β-amylase activity has been detected in chloroplasts from spinach leaves (23), several studies with other plants have indicated that most, if not all, of the cellular β-amylase activity is extrachloroplastic (13, 14, 18, 25, 26) and may be localized in the vacuole (15, 31). Precursors to the β-amylase of barley (11) and soybean (19) seeds and a precursor to the subunits of β-amylase in sweet potato tuberous roots (30) do not contain N-terminal transit peptide sequences that play a role in the transport of proteins into plastids. No β-amylase has been demonstrated to attack native starch granules that have not already been partially degraded by other enzymes or solubilized by boiling. Furthermore, varieties of soybean (1, 9) and rye (4) that exhibit greatly reduced levels of β-amylase activity in their seeds germinate normally. Thus, in spite of extensive enzymological studies of its amylolytic activity in vitro, the precise physiological role of β-amylase in plants is not known at present.

Tuberous roots of the sweet potato are unusually rich in β-amylase (2), and it represents one of the major proteins of the organ accounting for about 5% of the total soluble proteins. Only sporamin (17), which accounts for about 60 to 80% of the soluble proteins, is present at higher levels in these organs. Physicochemical and enzymological properties of the β-amylase from sweet potato have been studied extensively (for review, see ref. 28). Although the β-amylases from other plant and bacterial sources are monomeric enzymes with mol wt of about 50,000 to 60,000, the β-amylase from sweet potato is unique in that it is a tetramer of identical subunits, each with a mol wt of about 50,000 (28). In addition, the β-amylase from sweet potato has been shown to be identical to the starch phosphorylase-inhibitor protein that has been found in tuberous roots (22). However, the primary structure of the subunit of the β-amylase from sweet potato, as deduced from the nucleotide sequence of the corresponding cDNA, is not significantly different from those of the β-amylases of barley and soybean seeds (30).

The sweetness of tuberous roots of the sweet potato has been ascribed to the hydrolysis of starch to maltose by β-amylase during cooking, and the taste of varieties of sweet potato that have no or only low β-amylase activities is only slightly sweet when tuberous roots of these varieties are cooked (12). β-Amylase cannot be detected by immunological methods in some of the “nonsweet” varieties of sweet potato (K Oba, personal communication), yet these tuberous roots accumulate normal amounts of starch and support normal sprouting of the next generation, suggesting that β-amylase is not essential for the accumulation and mobilization of starch in the tuberous root.


In this report, we describe our finding that, while β-amylase polypeptides are not detectable or are present in only small amounts, in organs other than the tuberous roots of normal, field-grown sweet potato plants, the accumulation of a large amount of β-amylase occurs concomitant with the accumulation of starch and sporamin in leaf-petiole cuttings after the exogenous supply of sucrose or other metabolizable sugars. These observations suggest that the expression of the gene for β-amylase is under metabolic control which is coupled with the expression of storage function of the cells in the sweet potato.

MATERIALS AND METHODS

Plant Materials

Sweet potatoes (Ipomoea batatas Lam. var Kokei No. 14) were grown at the Nagoya University Experimental Farm. Petioles from plants with the intact leaf still attached were cut with a sharp razor blade. Five to ten of these leaf-petiole cuttings were combined and dipped in a solution of sucrose in water at the cut edge of the petioles, and they were then incubated under continuous light, unless otherwise indicated, at 25°C. Samples were either analyzed immediately or frozen in liquid nitrogen and stored at −80°C.

Extraction of Proteins and Analysis of β-Amylase and Sporamin

Proteins were extracted from tissues (7) and the protein content of each extract was determined, after precipitation with 10% TCA, by the method of Lowry et al. (16) with bovine serum albumin as a standard. SDS-PAGE and immunoblotting with sporamin-specific or β-amylase-specific antisera were carried out as described previously (7). Quantitation of the subunit of β-amylase in the extract was achieved by analysis of densitometric tracings of the immunoblots and amounts of sporamin were estimated by a dot-blot immunoassay. For activity staining of β-amylase, proteins in the extract were separated on 7% polyacrylamide gel containing 0.2% soluble starch. After electrophoresis, the gel was soaked in 0.2% soluble starch in 0.1 M sodium phosphate (pH 6.5) at 37°C for 1 h, stained for starch in 10 mM 1→4 mm KI in 1% acetic acid, and postfixed in 5% TCA-50% ethanol.

Determination of Starch

Tissues were pulverized in liquid nitrogen with the aid of a mortar and pestle, and the powder was suspended in 3 volumes (v/w) of ice-cold 10 mM Hepes-NaOH (pH 7.0). After grinding with a mortar and pestle, the homogenate was centrifuged at 20,000g for 15 min at 4°C. To remove soluble carbohydrates, the resultant precipitate was washed two times with cold 80% methanol and resuspended in water. The amount of starch was determined, after solubilization by heating in a boiling water bath for 2 h, with a starch-determination kit from Boehringer Mannheim.

Isolation and Analysis of RNAs

Total RNA was prepared from the tissue as described previously (6). RNA samples were denatured with formaldehyde and formamide, and then they were subjected to electrophoresis on 1.2% agarose gels that contained formaldehyde. Gels were then blotted onto Zeta probe nylon membranes (Bio-Rad). The membranes were hybridized with a 32P-labeled cDNA probe and washed essentially as described previously (6). The cDNA inserts from the following plasmids were used for hybridization: plMO23 (8), coding for A-type sporamin; pSPβA6, coding for the subunit of β-amylase from sweet potato (30); pFβ’A1, coding for the β’-subunit of mitochondrial F1-ATPas from sweet potato (unpublished results). After washing, membranes were either exposed to x-ray film (Kodak X-Omat) for autoradiography or each bands was subjected to a determination of radioactivity by a radioanalytical imaging system (Ambis, San Diego).

RESULTS

Localization of β-Amylase in the Sweet Potato

Proteins were extracted from leaves, petioles, stems, tuberous roots, and nontuberous roots of sweet potato plants. As shown in Figure 1 (top), the extract from the tuberous root contained several major proteins that cannot be seen in extracts of the other organs. The most heavily stained band, corresponding to a protein with an apparent mol wt of 25,000, represents sporamin (17). A protein with an apparent mol wt of 48,000 corresponds to the subunit of β-amylase. Immunoblot analysis of proteins from these organs showed that polypeptides that cross-reacted with β-amylase-specific antisera were detectable only in the extract from the tuberous root (Fig. 1, center). However, some preparations of extracts from nontuberous roots, stems and leaves contained very small amounts of β-amylase that was detectable by immunoblotting (data not shown).

β-Amylase in the extracts could be detected with greater sensitivity by separating proteins on nondenaturing polyacrylamide gels that contained soluble starch, with a subsequent assay for amyloytic activity in situ (activity staining; Fig. 1, bottom). The extract from the tuberous root showed a broad band of strong amyloytic activity around the region that corresponded to proteins with apparent mol wt of about 200,000. This band of amyloytic activity corresponds to the activity of β-amylase since the β-amylase purified from tuberous roots migrated to the same position and this band of amyloytic activity was absent in the extract from nontuberous roots of the β-amylase-null varieties of the sweet potato (data not shown). By contrast, only very weak amyloytic activity was detected in this region of the gel in extracts from other organs examined. These results indicate that in the normal, field-grown sweet potato plants, β-amylase is present in significant amounts only in tuberous roots, as is the case for sporamin (17). However, we have not examined the presence of β-amylase in other organs of the sweet potato such as flowers and seeds where relatively high β-amylase activities are present in some plant species (25, 27).

Induction of Accumulation of β-Amylase by Sucrose Occurs Concomitant with the Accumulation of Starch and Sporamin in Leaf-Petiole Cuttings

We showed previously that the accumulation of sporamin can be induced in leaves and petioles when leaf-petiole cut-
from petioles treated with 6% sucrose. The amount of the subunit of \( \beta \)-amylose in each extract was estimated by densitometric tracing of the immunoblot of the gel, and the amounts relative to the value obtained with extracts of petioles treated with 6% sucrose were plotted (Fig. 2). The relative amount of sporamin in the same extracts was also determined by dot-blot immunoassay of the extract with sporamin-specific antiserum. The greatest accumulation of sporamin was also obtained with 6% sucrose (Fig. 2).

We determined the amount of starch in the petiole samples used for the extraction of proteins. The accumulation of starch was also induced by sucrose, and the dependence of the accumulation on the concentration of sucrose was also similar to that in the case of \( \beta \)-amylose and sporamin (Fig. 2). The accumulation of these components was also observed in the leaf parts of the sucrose-treated leaf-petiole cuttings (data not shown; for sporamin, see ref. 6). These results indicate that all of the major biochemical markers of the tuberous root, namely, starch, sporamin, and \( \beta \)-amylose, can be induced to accumulate in leaf-petiole cuttings by an exogenous supply of sucrose, with similar responses to variations in the concentration of sucrose.

**Kinetics of Accumulation of \( \beta \)-Amylose, Sporamin, and Starch in Petioles after Treatment with Sucrose**

The kinetics of the accumulation of \( \beta \)-amylose, sporamin, and starch after treatment of leaf-petiole cuttings with various

![Figure 1. Detection of \( \beta \)-amylose in various organs of field-grown sweet potato plants. Equal amounts of protein (50 \( \mu \)g protein) in extracts from the leaf, petiole, stem, tuberous root, and nontuberous root of field-grown sweet potato plants were fractionated by electrophoresis on SDS-polyacrylamide gels and the gel was stained for protein with Coomassie brilliant blue (top, CBB) or analyzed by immunoblotting with antiserum against the \( \beta \)-amylose from sweet potato (center, Imm.). In addition, proteins were fractionated on a 7.5% polyacrylamide gel that contained soluble starch and assayed for amylolytic activity in situ (bottom, Activity). Numbers in parentheses indicate the amount of proteins applied to the gel.](image)

![Figure 2. Dependence of the accumulation of \( \beta \)-amylose, sporamin and starch in the petiole portions of leaf-petiole cuttings on the concentration of sucrose. Leaf-petiole cuttings were treated with a solution of sucrose at various concentrations for 7 d under continuous light. Proteins were extracted from the petiole portions of the cuttings and the relative amounts of \( \beta \)-amylose (●) and sporamin (○) polypeptides were determined. The relative amount of starch (□) was also determined with a portion of the same petioles as were used for the extraction of proteins. For \( \beta \)-amylose and sporamin, the relative amounts on the basis of equal weights of proteins are plotted with the value obtained with an extract of petioles treated with 6% sucrose taken as 1. For starch, the relative amounts on the basis of equal fresh weights of tissue are plotted.](image)
concentrations of sucrose were compared. The response of leaf-petiole cuttings to various concentrations of sucrose with respect to the kinetics and the extent of accumulation of these components fluctuated slightly from experiment to experiment, probably reflecting differences in the age and physiological condition of the explants. Figure 3 shows the result of one such experiment. Because leaves of cuttings treated with high concentrations of sucrose for a long time wilted severely, the experiment with a 9% solution of sucrose was carried out only once. The accumulation of β-amylase, sporamin, and starch in petioles treated with a 9% solution of sucrose for up to 7 d was similar to that in petioles treated with a 6% solution of sucrose (data not shown).

The patterns of accumulation of the three components in the petioles were not identical. In contrast to the accumulation of sporamin and starch, the amount of β-amylase accumulated in the petioles seemed to reach a plateau after about 1 week of treatment in most of the experiments. In contrast to the increase in the amount of sporamin, which required a lag period of a few days before significant increase to occur, the increase in the levels of β-amylase and starch did not show such a significant lag period. The accumulation of small amounts of each of these three components in petioles occurred when the cuttings were treated with water, and the induction obtained with a 1% solution of sucrose was not significantly different from that obtained with plain water (Figs. 2 and 3).

**Sucrose-Induced Accumulation of β-Amylase and Sporamin mRNAs**

The induction of the accumulation of sporamin in petioles after treatment with sucrose occurs at the level of mRNA (6). Total RNAs were prepared from the petiole portions of leaf-petiole cuttings treated with a 3% solution of sucrose or with water for 0, 1, 3, 5, and 7 d and analyzed for the levels of β-amylase and sporamin mRNAs by Northern blot hybridization. Unlike the case for sporamin mRNAs, we often detected small amounts of β-amylase mRNA in the RNA prepared from fresh, untreated petioles (Fig. 4). However, treatment of leaf-petiole cuttings with sucrose caused a dramatic increase in mRNAs for both β-amylase and sporamin. Accumulation of lower levels of β-amylase and sporamin mRNAs occurred in petioles treated with water. By contrast, neither electrophoretic patterns of RNAs after staining with ethidium bromide (data not shown) nor the level of mRNA for the nuclear-encoded δ'-subunit of mitochondrial F$_1$ATPase (Fig. 4) differed significantly among these preparations of RNA.

**Figure 3.** Time course of accumulation of β-amylase, sporamin and starch in the petiole portions of leaf-petiole cuttings after treatment with various concentrations of sucrose. Petiole portions of the cuttings were pulverized in liquid nitrogen and used for the extraction of proteins and the extraction of starch. The relative amount of β-amylase was determined by densitometric tracing of the immunoblot of an SDS-gel, and the relative amount of sporamin was determined by a dot-blot immun assay. The amount of starch was expressed as mg of starch per g of fresh tissue.

**Figure 4.** Analysis by Northern blot hybridization of mRNAs for sporamin and β-amylase in petiole portions of leaf-petiole cuttings. The gels were loaded with 20 μg each of total RNA from the petiole portions of leaf-petiole cuttings that had been treated with a 3% solution of sucrose or with water for 0, 1, 3, 5, and 7 d under continuous light. Filters were hybridized with cDNA probes specific for sporamin, β-amylase and F$_1$ATPase δ'-subunit. Exposure of filters was continued for 25 h for sporamin mRNA, 45 h for β-amylase mRNA, and 6 d for F$_1$ATPase δ'-subunit mRNA.
Radioactivity of $^{32}$P-labeled probes hybridized in each band, shown in Figure 4, was determined and the relative radioactivity of individual bands was plotted (Fig. 5; top panels). Lower panels of Figure 5 shows the results of a similar experiment with leaf-petiole cuttings treated with a 6% solution of sucrose for a shorter period. Both of the $\beta$-amylase and sporamin mRNAs could be detected as early as 6 h after the dipping of explants in the solution of sucrose, and the accumulation of $\beta$-amylase mRNA seemed to cease earlier than that of sporamin mRNAs.

Effects of Various Sugars on Induction

Leaf-petiole cuttings were treated with 3% or 6% solutions of various sugars for 4 d, and the accumulation of $\beta$-amylase in the petiole portions of the explants was analyzed by immunoblotting of SDS-polyacrylamide gels and by activity staining of non-denaturing polyacrylamide gels that contained soluble starch (Fig. 6). The accumulation of $\beta$-amylase was induced by treatment with 3% and 6% solutions of glucose or fructose. Mannitol did not cause an accumulation of $\beta$-amylase and sporamin, and it seemed actually to repress the accumulation of the small amount of $\beta$-amylase and sporamin that normally occurred during treatment with water alone. Sorbitol also did not cause any significant accumulation of $\beta$-amylase or sporamin above the accumulation observed with water. Minor amylolytic activities in the petals other than the $\beta$-amylase that were detected in activity-staining gels were not affected significantly by treatment of petioles with sucrose (data not shown).

Effect of Light on the Accumulation of $\beta$-Amylase

The sucrose-induced accumulation of $\beta$-amylase, sporamin and starch occurred in the absence of light (Fig. 7). Although treatment of leaf-petiole cuttings with water under continuous light induced the accumulation of small amounts of $\beta$-amylase, sporamin and starch (Fig. 3), the accumulation of these

![Sporamin mRNA](image)

![$\beta$-Amylase mRNA](image)

Figure 5. Changes in steady-state levels of mRNAs for sporamin and $\beta$-amylase in petiole portions of leaf-petiole cuttings after treatment with sucrose. Steady-state levels of mRNAs for sporamin and $\beta$-amylase were estimated by the determination of radioactivity of $^{32}$P-labeled probes hybridized after Northern blot hybridization of total RNA isolated from the tissue. The relative radioactivities on the basis of equal amounts of RNAs are plotted with the value which gave the highest level of radioactivity taken as 1. The top two panels show changes in the levels of mRNAs for sporamin and $\beta$-amylase in petioles after treatment with a 3% solution of sucrose (see Fig. 4), while the bottom two panels show those in petioles after treatment with a 6% solution of sucrose.
components after treatment with water was greatly reduced if the incubations were performed in darkness (data not shown; see Fig. 7 lane H2O). These results suggest that the endogenous supply of sucrose as a result of photosynthesis can induce the accumulation of small amounts of β-amylase, sporamin and starch in petioles, and that the sucrose-dependent induction of these components does not require light.

**DISCUSSION**

In contrast to the many studies on seed β-amylases, only a few studies on the regulation of the amount of β-amylase in vegetative tissues have been reported. Pongratz and Beck (23) reported that β-amylase activity in chloroplasts of spinach leaves shows a diurnal oscillation, with low activity during the day and high activity at night. In cotyledons of mustard, a phytochrome-mediated, light-induced increase in the synthesis of extra-chloroplastic β-amylase was observed (26). Caspar et al. (3) found that the amount of extra-chloroplastic β-amylase in leaves of *Arabidopsis thaliana* could be very high in mutants with altered starch metabolism, and they implied that β-amylase may be induced in response to the high levels of soluble sugars that accumulate during the photoperiod in the starchy mutants.

The results presented in this paper indicate that the expression of the gene for β-amylase is inducible in leaf-petiole cuttings of sweet potato plants by an exogenous supply of sucrose. Analysis of the nuclear gene for the subunit of β-amylase of sweet potato suggests that there may be only one copy per haploid genome of the gene that can hybridize with the cDNA (unpublished results). It is likely, therefore, that the β-amylase gene whose expression is induced in leaf-petiole cuttings by sucrose is identical to the one that is expressed in the tuberous root. In the case of sporamin, which is encoded by a multigene family, we showed previously that the same set of sporamin genes seems to be expressed in tuberous roots
and in petiole portions of sucrose-treated leaf-petiole cuttings (6).

The induction of the accumulation of β-amylase in leaf-petiole cuttings by an exogenous supply of sucrose occurred concomitant with the accumulation of sporamin and starch, and the accumulation of these three major components of the tuberous root in the leaf-petiole cuttings showed similar dependence on the concentration of sucrose (Fig. 2). Concomitant accumulation of β-amylase and sporamin was also induced by glucose or by fructose, but not by mannitol (Fig. 6). β-Amylase, sporamin and starch are accumulated within the same parenchymatous cells in the tuberous root of field-grown sweet potato plants (S. Takeda, Y. Kowyma, K. Nakamura, manuscript in preparation). Accumulation of large amounts of sporamin also occurs in stems of sweet potato plantlets cultured axenically on sucrose medium (7). The stems of these plantlets also contain large amounts of starch and β-amylase as well (our unpublished results). Taken together, these results suggest that the induction of the accumulation of β-amylase in leaf-petiole cuttings by sucrose occurs as part of the cellular expression of the vegetative storage functions, as normally exhibited by parenchymatous cells of the tuberous root. The fact that variable amounts of sporamin and β-amylase can occasionally be detected in organs other than the tuberous root in the field-grown plants suggests that, under certain physiological conditions, the temporal and simultaneous accumulation of sporamin, β-amylase, and starch occurs in certain cells of restricted area. Transgenic tobacco plants with a sporamin promoter-β-glucuronidase fusion gene express β-glucuronidase activity in cells that presumably function as a temporary sink (20).

A similar phenomenon to that discussed herein has been reported previously in potato plants by Paiva et al. (21), who observed that the accumulation of large amounts of the tuber storage protein, patatin, could be induced in stems and petioles of single-leaf stem cuttings concomitant with the accumulation of large amounts of other major tuber proteins, as well as starch. Results from several laboratories indicate that the expression of class I patatin genes of potato is also regulated by sucrose and that induction of these genes by sucrose accompanies the accumulation of starch (10, 24, 29). However, the concentrations of sucrose required to induce the accumulation of patatin in potato plants and that of β-amylase and sporamin in sweet potato plants are different. The maximum accumulation of patatin in leaf sections of potato plants requires sucrose at a concentration of 10% (10, 29), which is significantly higher than the concentration of sucrose that results in the maximum accumulation of β-amylase, sporamin and starch in the explants of sweet potato (Fig. 2).

The physiological role of the β-amylase that accumulates in the temporary and storage sink cells is not known. The precursor to the subunit of β-amylase in sweet potato does not contain a presequence at its N terminus (30), suggesting that β-amylase is localized outside of the plastids as are the β-amylases in vegetative tissues of other plants (13-15, 26, 30). Furthermore, β-amylase is unable to attack native starch granules without their prior digestion by other enzymes or solubilization by boiling (15, 27). Therefore, it is unlikely that β-amylase plays an essential role in the metabolism of transiently accumulated and reserve starch within the plastid.

Extrachloroplastic malto-dextrins have been suggested to be the substrates of β-amylase in vivo (15). However, the presence of such α-glucans needs to be established (3, 27), and the amount of β-amylase that is synthesized seems to be more than sufficient for such a role. As an alternative explanation, Giese and Heijgaard (5) suggested that β-amylase may have a nitrogen-storage function in barley seeds, since the profile of accumulation of β-amylase in developing seed resembles to that of the storage protein, hordein, and the synthesis of β-amylase responds to increased supply of nitrogen. Although β-amylase in the sweet potato may also participate in the temporary and long-term storage of nitrogen, this possibility does not rule out a possible role for β-amylase in the metabolism of glucans. Further analysis of the regulation of expression of the gene for β-amylase in the sweet potato should shed light on the physiological role of this enzyme.

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LITERATURE CITED

SUCROSE INDUCES SWEET POTATO $\beta$-AMYLASE


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